

A versatile MALDI target plate based on polymer-filled reticulated vitreous carbon foam

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Introduction

High-resolution, 3-dimensional MALDI-TOF plates¹ have been developed as a potential interface between PAGE separations and tissue samples with MALDI mass spectrometry. The target plate is composed of a Duocel® reticulated vitreous carbon (RVC) foam² and polymer monoliths. The RVC foam provides a robust, porous, electrically conductive scaffold and the porous polymer monoliths provides a substrate for capture and concentrate the peptides or proteins.

Methods

Plate Construction

- Duocel® RVC sheets (2mm thick) were purchased from ERG Materials and Aerospace Corp
- Porous styrene-based monoliths synthesized within RVC sheets in an in-house reaction chamber
- Post polymerization, plate is shaved to flatness with a razor blade
- Protein and peptides samples may be applied to plate by any means
- Loaded sample may be washed as needed and is then eluted to to one surface with MALDI matrix and analyzed by MALDI-TOF

Polymer Monolith

- styrene/divinylbenzene construction
- thermal initiation
- in house reaction chamber
 - rotates to help insure consistent plate construction
- polymer cleaned to be coincident with RVC substrate using razor blade

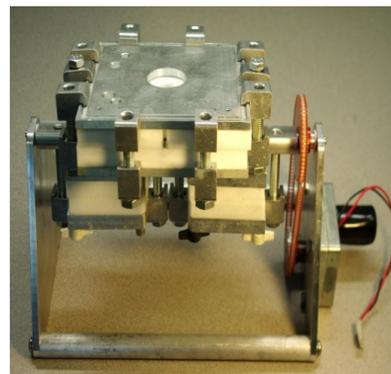
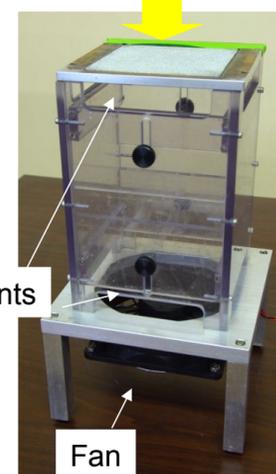


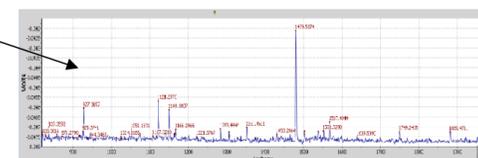
Plate Elution

- Analyte washing and elution occur on in-house designed elution chamber
- elution solvent applied on top surface and evaporated off bottom surface
- evaporation augmented by use of fan to reduce vapor pressure
- matrix is added to solvent to produce MALDI crystals on bottom surface
- Post elution, sample is analyzed in MALDI-TOF mass spec

Elution solvent containing matrix

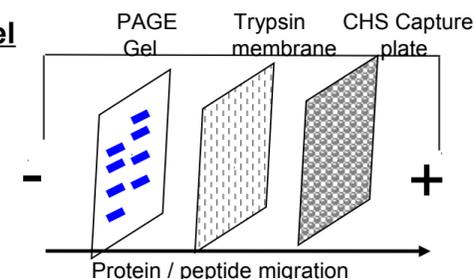


Eluted section showing dried MALDI matrix



Results

Interface with PAGE Gel

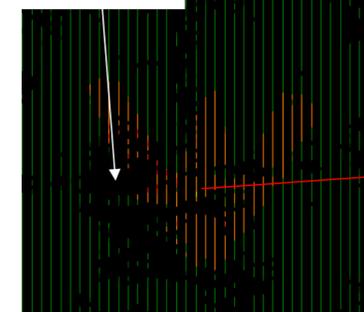


- Based on "molecular scanner"³ concept
- PAGE separated protein blotted through digestion membrane and captured and concentrated on RVC polymer plate
- plate is washed eluted and analyzed by MALDI-TOF mass spec

Preliminary Studies

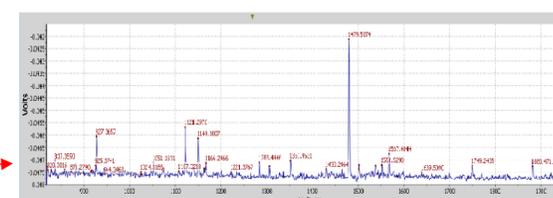
- preliminary studies have focused on optimization of sample elution using blots of pre-digested protein (BSA)
 - optimization of wash and sample elution to plate surface
 - quantities, times and formulation
 - optimization of matrix formulation and concentration
 - optimization of controlling parameters
 - air flow, suction

Incomplete elution



TIC of BSA peptide (1479.74) orange and ACHC matrix dimer (379.09) green

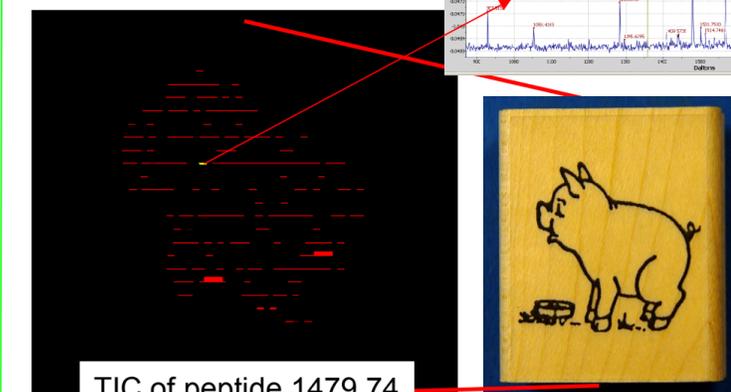
Blot of BSA peptides in the shape of a "W"



Spectrum from central pixel showing BSA peptides

Tissue Imaging

Image of stamped pig



TIC of peptide 1479.74

- it is envisioned that the RVC-polymer plate may serve as a substrate for tissue impression
- polymer substrate may adsorb analyte (protein, peptide, lipid) from biological tissue and preserve spatial resolution
- analyte washing, elution and detection will occur as described

Ongoing Studies

- continued optimization of elution parameters
- construction and optimization of digestion membrane
- optimization of blotting condition to maximize analyte transfer, digestion and capture
- preliminary study with biological tissue

References

- 1)Hattan SJ, Vestal ML (2008) Anal Chem.; 80 : 9115-9123
- 2) ERG Materials and Aerospace Corp. 900 Stanford Avenue Oakland, CA 94608
- 3) Nadler TK, et al. (2004) Anal. Biochem.; 332 : 337-348.

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